

# Genotype $\times$ environment interactions for herbage yield of perennial ryegrass sward plots in Ireland

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## Abstract

Perennial ryegrass (*Lolium perenne* L.) is by far the most widely sown grass species in Ireland. Genotype  $\times$  environment ( $G \times E$ ) interactions are a frequent occurrence in herbage yield evaluations. The objectives were to determine (i) the nature and relative magnitudes of the pertinent  $G \times E$  interaction variance components for dry matter yield of perennial ryegrass sward plots in Ireland and (ii) the optimal allocation of replicates, locations and years in a testing programme. Sixteen perennial ryegrass cultivars were sown at six locations throughout Ireland between 2000 and 2004. Plots from each sowing were harvested for 2 consecutive years under a simulated mixed grazing and conservation management programme. The largest component of the  $G \times E$  variance was generally genotype  $\times$  location  $\times$  year emphasizing the need for evaluation of genotypes across locations and years to adequately characterize genotypes for differences in yield. Relative differences among genotypes from year to year and location to location were due mainly to changes in genotype rankings. Weather was estimated to have a greater effect on annual variation in herbage yield than age of stand. The optimum allocation of resources for a testing programme was estimated at four replicates per location, and either two locations and 3 sowing years or three locations and 2 sowing years with 2 harvest years for each sowing year. The most appropriate option depends on the relative importance of time vs. financial resources.

**Keywords:** perennial ryegrass, forage yield, genotype  $\times$  environment interactions, variance components, resource allocation

## Introduction

Irish agriculture is overwhelmingly grass-based with 0.811 of agricultural land under grass pasture (Drennan *et al.*, 2005). The most widely sown grass species is perennial ryegrass (*Lolium perenne* L.), accounting for about 0.95 of agricultural grass seed sales. Of this total, intermediate- and late-maturing cultivars predominate, while diploid cultivars account for about two-thirds of seed sold (Department of Agriculture and Food, personal communication).

Regional and annual variation in grass production in Ireland is of great significance. In an average year the length of the grass-growing season varies from about 8 months in the north-east to 11 months in the south-west. Model estimates of annual dry matter (DM) yield range from about 11 t ha<sup>-1</sup> in the north-east to 15 t ha<sup>-1</sup> in the south-west (Brereton, 1995). Dry matter yield range, omitting extreme years, is approximately 0.20 of the mean in the January to April and in the September to December periods, while in the May to August period it is about 0.10 (Brereton, 1984). Such instability suggests considerable environmental variation across locations and years as dictated by climatic, soil and biotic conditions. As perennial ryegrass is a perennial crop, year-to-year variation may also constitute age-of-stand effects.

Genotype  $\times$  environment ( $G \times E$ ) interactions are a frequent occurrence in herbage yield evaluations of perennial ryegrass sward plots (Talbot, 1984; Posselt, 1994; Jafari *et al.*, 2003). This indicates changes in the rank order of genotypes (crossover interactions), the magnitude of differences among genotypes (non-crossover interactions) or both, from one environment to another. If herbage DM yield is the sole trait under consideration, the only  $G \times E$  interactions of practical significance to breeders and agronomists are crossover interactions. If crossover interactions do not exist, genotypes that are superior in one environment maintain their superiority in other environments. However, if as is more often the case, a range of traits are under simultaneous consideration, the magnitude of

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differences among genotypes is equally important as differences in yield, depending on their extent, may be compensated for by improved performance for other traits.

Because of the relatively small land base and grass seed market in Ireland, economically viable cultivars must express wide adaptation over the whole country. Cultivars must also perform over an extended duration of time with reseeded swards expected to last for at least 5 years and usually much longer (> 10 years). Most Irish grassland consists of permanent swards, with only about 0.03 of the total area reseeded each year (Keating and O'Kiely, 2000). Thus, if  $G \times E$  interactions occur, it is necessary to evaluate cultivars across a range of environments to estimate their true value.

Yield trials are both costly and time-consuming. To maintain cost-effectiveness, breeders and agronomists need to define the minimum number or optimum combination of replicates, locations and years necessary to adequately measure the genetic value of a genotype. The available resources must be used in a manner which minimizes the error in assessing the merit of the test material while recognizing the realities of environmental interactions.

Replication within a location provides a mechanism to improve the precision of measurements and the power to discriminate genotypes at that location. However, using more replicates at a single location will not provide additional information on genotype performance across the target region if  $G \times E$  interactions exist between the test and target regions. Hence, for a fixed total number of plots per genotype, better discrimination among genotypes would be obtained by adding locations rather than replicates (Bernardo, 2002). The expense of shifting to more locations with fewer replicates per location, however, can be prohibitive. The ideal location, or combination of locations, should reflect the relative potential of genotypes over the target region and maximize genetic variation, and hence response to selection (Allen *et al.*, 1978).

If, as is often the case, the three-factor genotype  $\times$  location  $\times$  year interaction is found to be the largest source of  $G \times E$  interaction for herbage yield, testing over locations and years is necessary. For perennial crops, year-to-year weather and age-of-stand effects are confounded within year. However, repeated sowings may allow inferences to be gained on the separate effects of both these components. Genotype  $\times$  year-to-year weather interaction effects are unpredictable and cannot be exploited in future years, as the climatic conditions that generate year-to-year weather variation are not known in advance. However, provided variation among genotypes over years exists, a broader environmental inference will be gained by testing over a number of years. Given that perennial

ryegrass swards are normally harvested successively through each of several years, a differential change in yields of genotypes as the stand ages suggests that repeated testing of trials over a number of harvest years may have a significant impact on genotype means over time.

Knowledge of the nature and relative magnitudes of the various types of  $G \times E$  interactions is necessary to determine the optimum allocation of resources in a testing programme. Although regional cultivar trials have been a part of the national perennial ryegrass testing programme in Ireland for many years, the  $G \times E$  interaction effects have not been documented. The objectives were to determine (i) the nature and relative magnitudes of the pertinent  $G \times E$  interaction variance components for herbage DM yield of perennial ryegrass sward plots in Ireland and (ii) their implications for the optimal allocation of replicates, locations and years in a testing programme.

## Materials and methods

### Germplasm and management

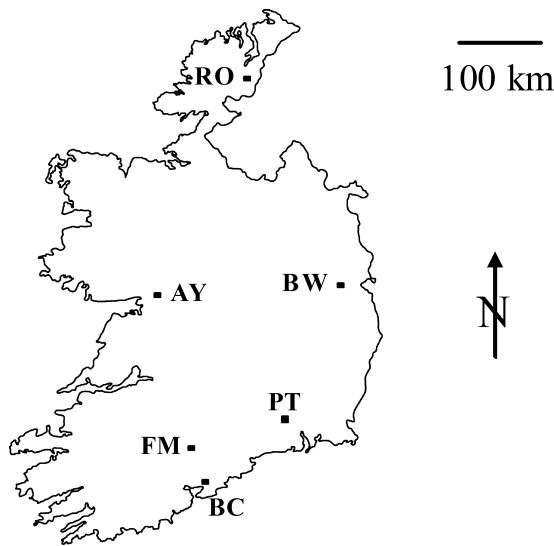
Perennial ryegrass cultivar trials were sown at six locations throughout Ireland between 2000 and 2004 (Table 1 and Figure 1). The locations were Athenry, Co. Galway (AY), Ballinacurra, Co. Cork (BC), Backweston, Co. Kildare (BW), Fermoy, Co. Cork (FM), Piltown, Co. Kilkenny (PT) and Raphoe, Co. Donegal (RO). Weather data, recorded at meteorological stations near each location during harvest years 2001–2006, are presented in Figure 2. Intermediate- and late-maturing cultivars were arranged in separate trials and, with one exception, sown at each sowing year-location combination. Eight cultivars were common across all intermediate- and late-maturing trials and were considered to adequately represent the germplasm pool available to farmers in Ireland (Table 2). These cultivars (hereafter called genotypes) accounted for 0.62 of intermediate and late perennial ryegrass cultivars recommended for agricultural use in Ireland in 2004 by the Irish government (Department of Agriculture and Food, 2004).

The experimental design of each trial was a randomized complete block with three blocks (sowing year 2000) or four blocks (sowing years 2001 to 2004). Plots (7.0 m  $\times$  1.5 m) were sown in late summer as pure swards, following full cultivation, at a rate of 31 and 41 kg ha<sup>-1</sup> for diploid and tetraploid cultivars respectively. Extra plots were sown at the ends of each row to reduce border effects. During the establishment year, plots were sprayed with the herbicides mecoprop-P (48.3% w/w) (Duplosan KV, Nufarm UK Ltd., Kent, UK) to control broad-leaved weeds and ethofumesate (44.3% w/w) (Nortron Flo, Bayer CropScience Ltd.,

**Table 1** Location and sowing year information for intermediate- and late-maturing perennial ryegrass cultivar trials conducted in Ireland between 2000 and 2006.

Location					
Name	Code	N latitude	W longitude	Altitude (m)	Sowing years
Athenry	AY	53°18'	8°45'	35	2001, 2002, 2003, 2004
Ballinacurra	BC	51°54'	8°10'	20	2000
Backweston	BW	53°22'	6°30'	50	2000, 2001, 2002, 2003, 2004
Fermoy	FM	52°08'	8°17'	53	2003, 2004
Piltown	PT	52°21'	7°20'	15	2001, 2002, 2003, 2004
Raphoe	RO	54°52'	7°36'	65	2000, 2001, 2002, 2003, 2004

\*Late-maturing cultivars not sown in 2004 at location AY.

**Figure 1** Geographic distribution of the six trial locations in Ireland. See Table 1 for location codes.

Cambridge, UK) to control annual meadow grass. Plots were cut at 4- to 6-week intervals according to local growth conditions, but no measurements were taken. Plots were limed and fertilized according to soil-test recommendations.

For 2 consecutive years thereafter, plots were harvested six times per year. The cutting regime was designed to mimic a mixed grazing and forage-conservation management. The first harvest (Cut 1; early spring growth) was taken in early April followed by two conservation-stage cuts at regrowth intervals of 7 (Cut 2; first cut silage) and 6 (Cut 3; second cut silage) weeks. A further three simulated grazing cuts were taken at regrowth intervals of 4 (Cut 4; mid-summer growth), 5 (Cut 5; late-summer growth) and 6 (Cut 6; early-autumn growth) weeks. Herbage yield was determined by harvesting the entire plot to a stubble height

of 5 cm above ground level using a plot harvester (Haldrup, Lögstör, Denmark). Random herbage samples of c. 300 g were dried at 80°C for 16 h to determine the DM content and adjust plot yields to a DM basis. Dry matter yields for each plot were summed across all harvests within each year to give the annual herbage DM production for a given plot.

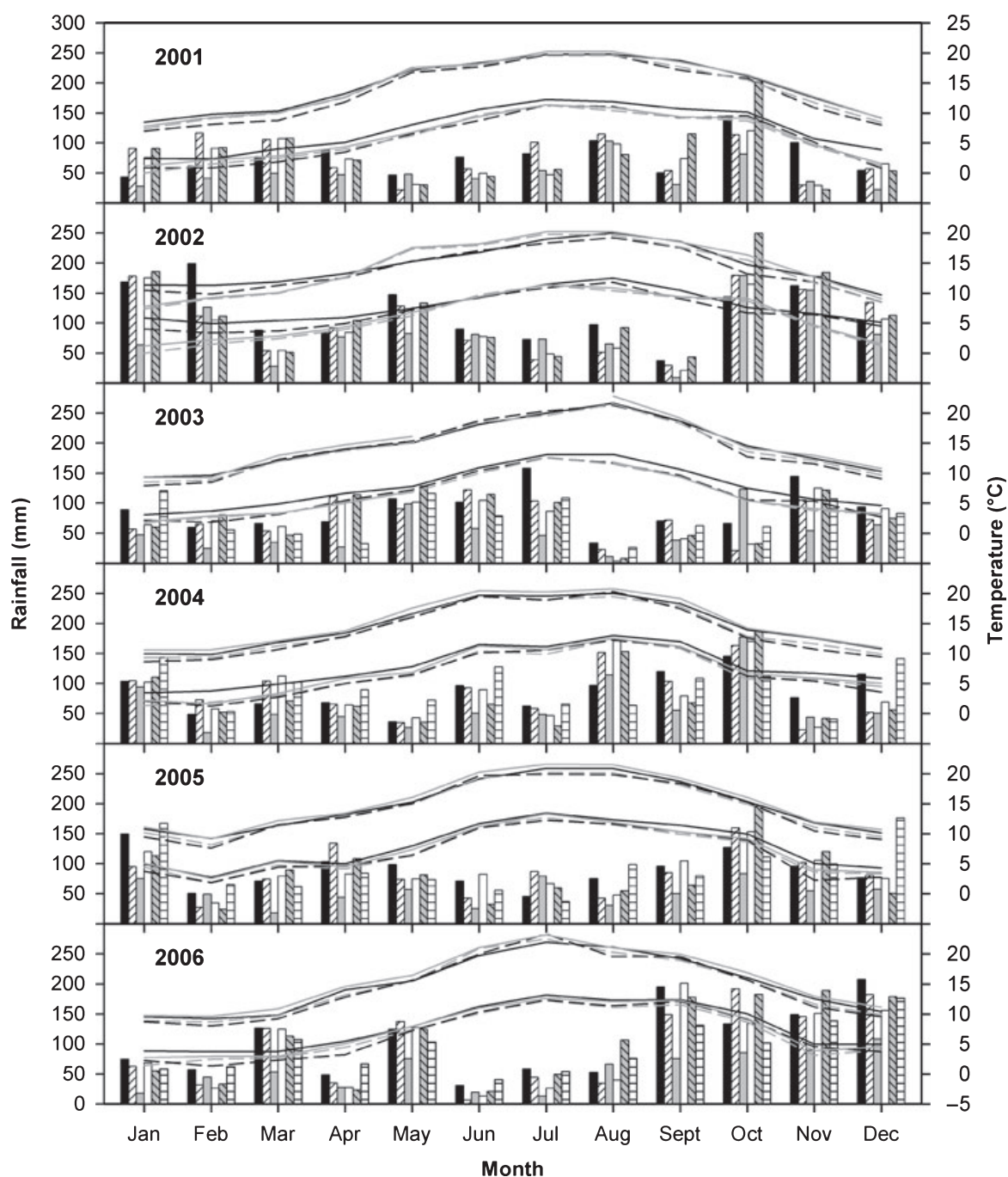
Nitrogen fertilizer was applied as split dressings in mid-February and after all but the final harvest each year to give an annual total of 295–400 kgN ha<sup>-1</sup>. For each sowing year-harvest year (first- or second-harvest year) combination, all locations received identical rates of N fertilizer. During only one calendar year were differential rates of N fertilizer (295 vs. 350 kg N ha<sup>-1</sup>) applied to first- and second-harvest-year swards. Fertilizer P as P<sub>2</sub>O<sub>5</sub> was applied after Cut 6 in the first-harvest-year at 24.5 kg ha<sup>-1</sup>. Fertilizer K was applied as K<sub>2</sub>O after Cut 2 (150 kg ha<sup>-1</sup>) and Cut 6 (180 kg ha<sup>-1</sup>) in the first-harvest year and after Cut 2 (150 kg ha<sup>-1</sup>) in the second-harvest year.

### Statistical analyses

Statistical analyses were conducted using the SAS statistical package (SAS Institute Inc, Cary, NC, USA). The results were pooled across intermediate- and late-maturity categories. Information on the genotype and G × E interaction effects was obtained from an analysis of variance on the combined data (six locations and 5 sowing years each with 2 harvest years nested within the two maturity categories) using the model

$$\begin{aligned}
 Y_{ijklmn} = & \mu + M_m + L(M)_{lm} + B(LM)_{klm} + A(M)_{jm} \\
 & + Y(AM)_{jmn} + LA(M)_{jlm} + LY(AM)_{jlmn} \\
 & + YB(LM)_{lkmn} + G(M)_{im} + GL(M)_{ilm} \\
 & + GB(LM)_{iklm} + GA(M)_{ijm} + GY(AM)_{ijmn} \\
 & + GLA(M)_{ijlm} + GLY(AM)_{ijlmn} + \varepsilon_{ijklmn} \quad (1)
 \end{aligned}$$

where  $\mu$  = the grand mean,  $M_m$  = the effect of maturity category,  $L_l$  = the effect of location,  $B_k$  = the effect of



**Figure 2** Total monthly rainfall (black solid column, location AY; white column with diagonal lines, location BC; grey solid column, location BW; white solid column, location FM; grey column with diagonal lines, location PT; white column with horizontal lines, location RO) and mean monthly minimum and maximum temperatures (black solid line, location BC; black dashed line, location BW; grey dashed line, location FM; grey solid line, location PT) recorded at local meteorological stations during years 2001 to 2006. Note missing data: rainfall at RO for years 2001 and 2002, and minimum and maximum temperatures at AY and RO for all years and at PT for June and July 2003.

**Table 2** Description of the sixteen perennial ryegrass cultivars evaluated (Department of Agriculture and Food, 2004).

Maturity category	Cultivar <sup>†</sup>	Ploidy	Heading date	Breeder	Country of origin
Intermediate	Spelga	Diploid	18 May	DARD	UK
	Cashel	Diploid	19 May	Teagasc	Ireland
	Magician	Tetraploid	19 May	Teagasc	Ireland
	Napoleon	Tetraploid	20 May	DLF Trifolium	Denmark
	Aubisque	Tetraploid	21 May	Advanta	The Netherlands
	Respect	Diploid	23 May	Cebeco	The Netherlands
	Premium	Diploid	24 May	Cebeco	The Netherlands
	Greengold	Tetraploid	25 May	Teagasc	Ireland
Late	Gilford	Diploid	01 June	DARD	UK
	AberCraigs	Tetraploid	02 June	IGER	UK
	Tyrone	Diploid	03 June	DARD	UK
	Portstewart	Diploid	06 June	DARD	UK
	Millennium	Tetraploid	08 June	Teagasc	Ireland
	Mammout	Tetraploid	09 June	Advanta	The Netherlands
	Cancan	Diploid	10 June	Limagrain	France
	Sarsfield	Tetraploid	11 June	Teagasc	Ireland

<sup>†</sup>Yield data provided by Department of Agriculture and Food, Ireland.

block,  $A_j$  = the effect of age of stand,  $Y_n$  = the effect of sowing year,  $G_i$  = the effect of genotype and  $\epsilon_{ijklmn}$  = the residual error. Age of stand represented the number of harvest years after sowing and its levels were 1 or 2 years of age. Maturity category and age-of-stand effects were considered to be fixed. Genotype, block, location, sowing year and all interaction effects were considered to be random. Genotype effects were considered random because they were representative of the population of cultivars marketable in Ireland. Location effects were considered random because they were representative of fertile, productive and well-managed grassland sites, and hence the predominantly reseeded sites, within the target region. Variance components were estimated by equating mean squares to their expectations (Gaylor *et al.*, 1970). Negative components of variance were considered as zero.

Because the combined analysis of variance described in Eqn (1) indicated the presence of significant  $GLY(AM)_{ijlmn}$  interactions, each combination of location, sowing year and age of stand was considered a separate macro-environment. An analysis of variance was performed using the model

$$Y_{ijkl} = \mu + M_j + E(M)_{jl} + B(EM)_{jkl} + G(M)_{ij} + GE(M)_{ijl} + \epsilon_{ijkl} \quad (2)$$

where  $\mu$  = the grand mean,  $M_j$  = the effect of maturity category,  $E_l$  = the effect of macro-environment,  $B_k$  = the effect of block,  $G_i$  = the effect of genotype and  $\epsilon_{ijkl}$  = the residual error. All effects were considered to be random, except maturity category. Joint regression analysis was used to study the  $GE(M)_{ijl}$  effect (Freeman and Perkins, 1971). An environmental index

was calculated for each combination of maturity category and macro-environment as the mean value of all intermediate- or late-maturing genotypes in that environment. Genotype values within an environment were regressed against the corresponding environmental indices and the  $GE(M)_{ijl}$  effect was partitioned into one component of linear regressions onto the environmental index (heterogeneity of regression) and another of deviation of regressions (residual). Variance components were estimated by equating mean squares to their expectations (Gaylor *et al.*, 1970). Negative components of variance were considered as zero.

It was assumed that the principal differences between first- and second-harvest-year data, when harvested at the same location in the same calendar year, were due to age-of-stand effects. Other effects could include fertility differences, soil-type variation and soil moisture differences. However, these effects were considered to be negligible compared to age-of-stand effects, because comparable-age trials were located < 500 m apart on the same uniform soil type, had a similar cropping history and received similarly high rates of fertilizer. Weather effects were estimated by comparing swards of similar age at the same location, but tested in different calendar years. Obvious weather effects would be due to differences in light, temperature, moisture and fertility between the specific years under test, but would also include accumulated or carryover effects from the time of establishment, in-so-far-as they were important. Location effects were estimated by comparing swards of similar age tested at different locations in the same calendar year. Using these criteria, all possible pairs of first- and second-year harvests, year-to-year

weather effects and locations were calculated for each maturity category on the basis of genotype means across all blocks. The level of crossover interactions for each variable was determined using Spearman's rank correlation coefficients between genotype arrays. Rank correlation coefficients were pooled across maturity categories.

Optimum allocation of resources was based on the predicted least significant difference (LSD) for testing the difference between the means of two genotypes, calculated for various levels of replicates, locations and sowing years, each with 2 harvest years (age of stand = 2), as

$$LSD = t_{(0.05/2)} \times \left[ 2 \times \left( \frac{\sigma_{GL/M}^2}{lm} + \frac{\sigma_{GB/LM}^2}{blm} + \frac{\sigma_{GA/M}^2}{am} + \frac{\sigma_{GY/AM}^2}{yam} + \frac{\sigma_{GLA/M}^2}{lam} + \frac{\sigma_{GLY/AM}^2}{lyam} + \frac{\sigma_e^2}{blyam} \right) \right]^{\frac{1}{2}}$$

where  $t_{(0.05/2)}$  is the two-tailed  $t$ -value at the 0.05 significance level;  $\sigma_{GL/M}^2$ ,  $\sigma_{GB/LM}^2$ ,  $\sigma_{GA/M}^2$ ,  $\sigma_{GY/AM}^2$ ,  $\sigma_{GLA/M}^2$  and  $\sigma_{GLY/AM}^2$  are the estimated variance components associated with  $G \times E$  interactions, and  $\sigma_e^2$  the error variance, derived from the combined analysis of variance described in Eqn (1); and  $l$ ,  $m$ ,  $b$ ,  $a$  and  $y$  are the number of locations, maturity categories, blocks, age-of-stand years and sowing years, respectively. The  $t$ -value was assumed to be 2.0 for all calculations given that the number of genotypes to be tested will usually be large (Bernardo, 2002). The LSD was expressed as a percentage of the grand mean for that harvest period, providing a relative measure of the extent to which genotype differences can be detected.

Optimum testing locations were assessed using the term  $r_P\sqrt{H}$  described by Allen *et al.* (1978). Values of  $r_P\sqrt{H}$  were calculated separately for each maturity category but pooled in the presentation of the results. The coefficient ( $r_P$ ) is the phenotypic correlation between genotype means for each sowing year-location environment and the estimate of genotype means over all environments, i.e. the best estimate of the true mean genotype performance. The term  $H$  is defined as broad sense heritability [equivalent to repeatability as described by Falconer and Mackay (1996)] and was calculated for each sowing year-location environment as

$$H = \frac{\sigma_G^2}{\sigma_G^2 + \left( \frac{\sigma_{GB}^2}{b} \right) + \left( \frac{\sigma_{GA}^2}{a} \right) + \left( \frac{\sigma_e^2}{ab} \right)} \quad (4)$$

where  $\sigma_G^2$ ,  $\sigma_{GB}^2$ ,  $\sigma_{GA}^2$  and  $\sigma_e^2$  are the estimated variance components for genotype, genotype  $\times$  block, genotype  $\times$  age of stand and error, respectively, and  $b$  and  $a$  are the number of blocks and age-of-stand years respectively. Negative estimates of  $H$  were considered as zero.

## Results and discussion

### Maturity category

Cultivars are grouped into arbitrarily designated maturity categories based on heading date mainly for ease of management of trials. In reality, heading date represents a continuum such that there may be little difference between the latest maturing cultivar in one group and the earliest maturing in the next. Classifying cultivar performance simply based on their maturity label can be misleading (Gilliland, 2006). The reference population in the current study was all marketable perennial ryegrass cultivars in Ireland, throughout the continuum of heading date. Early-maturing cultivars were not considered as they account for less than 0.001 of perennial ryegrass seed sales in Ireland (Department of Agriculture and Food, personal communication). Separate analyses for intermediate- and late-cultivar trials (data not presented) indicated homogenous results across maturity categories. Based on the statistical evidence and considering maturity to exist along a continuum, the results, as described below, were pooled across intermediate- and late-maturity categories. In a comparable study estimating the magnitude of  $G \times E$  interactions for herbage DM yield in the UK national perennial ryegrass trials, Talbot (1984) also analysed the data across maturity categories.

### Variance component estimates

Genotypes varied ( $P < 0.05$  or less) for mean DM yield for all harvest periods, but their interactions with environmental factors were highly complex (Table 3). This illustrates the difficulties encountered by breeders and agronomists in selecting genotypes on account of the masking effects of variable environments. The combined  $G \times E$  ( $\sigma_{GL/M}^2 + \sigma_{GB/LM}^2 + \sigma_{GA/M}^2 + \sigma_{GY/AM}^2 + \sigma_{GLA/M}^2 + \sigma_{GLY/AM}^2$ ) variance was typically large relative to the genotype variance ranging from 0.51 to 4.13 depending on harvest, with an overall mean of 2.12. As is the case with many  $G \times E$  interaction studies (Talbot, 1984; Posselt, 1994), the largest component of the  $G \times E$  variance was generally  $\sigma_{GLY/AM}^2$ . This indicates that genotype  $\times$  location effects were inconsistent from year to year, independent of age of stand, or that genotype  $\times$  year-to-year weather effects were inconsistent from location to location. It was assumed that, for a given age of stand, differences among years were primarily due to direct and indirect weather effects. As is the nature of Ireland's climate (Keane and Sheridan, 2004), there was significant monthly variation in rainfall between years and local variation within months in individual years (Figure 2). Corresponding variation in minimum and

**Table 3** Estimates of genotype and genotype  $\times$  environment components of variance for herbage yield of perennial ryegrass cultivars (t dry matter ha<sup>-1</sup>  $\times$  10<sup>3</sup>).

Source of variation†	Harvest						
	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	Cut 6	Annual
G	6.4*	69.6***	55.6***	1.2*	4.6***	2.1***	60.1**
G $\times$ L	2.1*	0.0§	0.6	0.3	2.4**	1.3***	4.9
G $\times$ B/L	0.0	0.0	2.3	0.3	1.4*	0.1	48.7***
G $\times$ A	5.7***	3.4	2.3	0.3	1.2*	0.3	22.2*
G $\times$ Y/A	2.4**	6.2	3.8‡	0.1	0.0	0.0	0.0
G $\times$ L $\times$ A	1.6‡	9.1	0.0	0.0	0.0	0.0	12.7
G $\times$ L $\times$ Y/A	4.1**	27.2***	19.3***	4.2***	5.2***	2.1***	81.3***
Error	50.7	333.3	186.8	37.4	50.2	25.4	818.9

†G, genotype; L, location; B, block; A, age of stand; Y, sowing year; all terms are nested within maturity category.

‡, \*, \*\* and \*\*\* indicate  $P < 0.10$ ,  $0.05$ ,  $0.01$  and  $0.001$ , respectively, of the appropriate mean squares in the analysis of variance.

§Negative estimates are given as zero.

maximum temperatures was comparatively small (Figure 2).

When  $G \times E$  interaction arise from variation in unpredictable environmental factors, such as year-to-year weather effects, as in this study, the breeder or agronomist must select genotypes that can perform reasonably well over a range of conditions. This emphasizes the need for evaluation of genotypes across both locations and years to adequately characterize genotypes for differences in DM yield. It is not sufficient to concentrate on only the spatial or temporal aspects of environmental variation. Although this gives no indication as to how testing should be distributed through time and space, the magnitudes of  $\sigma^2_{GLA/M}$  and  $\sigma^2_{GY/AM}$  indicate that locations and years contributed a similar proportion to the  $G \times E$  interaction structure. Additional locations can be readily substituted for years in a testing programme, and *vice versa*, without any significant effect on the true estimate of genotype DM yield.

Age of stand was also a relatively large contributor to the  $G \times E$  variance. Camlin and Stewart (1975) found that cultivar persistence had a major influence on production as the sward ages. Therefore, an optimal testing procedure would incorporate both repeated sowings and repeated measurements within sowings (Casler, 1999a). However, the magnitude of  $\sigma^2_{GLY/AM}$  was, in general, significantly greater than  $\sigma^2_{GLA/M}$  indicating that weather variation was more important than age of stand in influencing genotype  $\times$  year interactions. Therefore, increasing the number of harvest years per sowing may offer a more cost-effective means to characterize a genotype than maintaining only 2 harvest years for an increasing number of sowing years. Casler (1999a) reported that each year of data, from the first to third harvest year, provided an incremental gain in rank correlation for annual DM yield of perennial ryegrass sward plots. Meaningful changes in rank

correlations with increasing age suggest that there is benefit in harvesting trials beyond the second production year. Testing beyond 2 harvest years would also provide a more severe test of persistence. Gilliland and Mann (2000) reported that differences in perennial ryegrass cultivar rankings between forage conservation and simulated grazing managements were only observed in the third harvest year.

The large variance component for the interaction of genotype  $\times$  block nested within location–maturity category combination for annual DM yield indicates that the magnitude of the annual DM yield differences among genotypes varied from block to block at the individual locations. As seed from a single seed lot was used to establish plot replicates, the yield variability from such replicates should be due almost entirely to submacro- to micro-environmental variability, experimental error, or both. The large error variance is not unusual even in research-station trials (Talbot, 1984). The relative size of both these effects could be reduced by using more uniform test environments, more replicates, or both. Although annual production is commonly presented as an index of genotype performance, it is, in practice, of limited value as the economic value of extra grass production can vary enormously throughout the growing season (Doyle and Elliott, 1983). In this regard, increasing the number of replicates solely to improve the precision of annual DM yield measurements would not be recommended. The use of more elaborate experimental designs, spatial analyses, or both may prove beneficial in reducing experimental error (Casler, 1999b).

### Joint regression analysis

Joint regression analysis indicated that the heterogeneity of regressions were generally not significant and the

**Table 4** Joint regression analysis of variance for genotype yield values on the macro-environment mean yield (t dry matter ha<sup>-1</sup> × 10<sup>3</sup>).

Source of variation†	Harvest						
	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	Cut 6	Annual
Environment (E)	463.2***	1478.2***	1311.5***	418.0***	502.1***	206.1***	6690.9***
Block / E	23.9***	56.6***	49.6***	14.0***	14.6***	9.0***	228.0***
Genotype (G)	9.9***	71.6***	56.9***	1.3***	5.6***	2.3***	74.2***
G × E	16.1***	47.5***	27.1***	5.3***	7.4***	2.8***	128.2***
Heterogeneity of regressions	0.8**	2.9*	1.3*	0.0§	0.1	0.0	2.7
Residual	15.2***	44.7***	25.8***	5.3***	7.4***	2.8***	125.5***
Error	36.0	297.4	166.1	31.6	46.2	21.3	785.2

†All terms are nested within maturity category; the variance for the main effect of maturity category was not estimated as it was considered to be fixed.

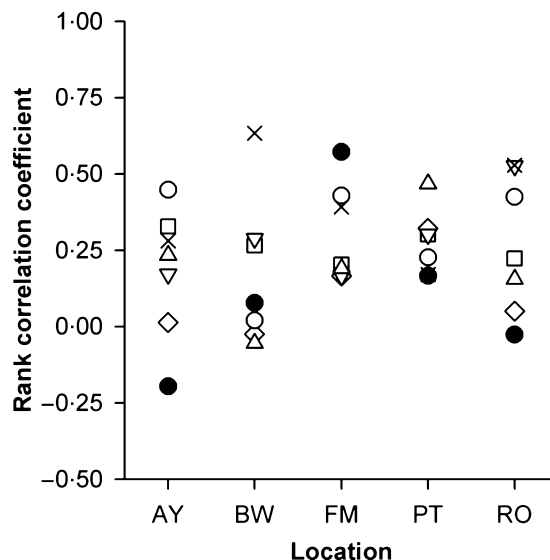
\*, \*\* and \*\*\* indicate  $P < 0.05$ ,  $0.01$  and  $0.001$ , respectively, of the appropriate mean squares in the analysis of variance.

§Negative estimates are given as zero.

proportion of the  $G \times E$  interaction due to linear regression was relatively low, ranging from 0 to 0.06 (Table 4). The residuals of the  $G \times E$  interaction were all highly significant ( $P < 0.001$ ). Thus, a major proportion of the  $G \times E$  interaction was not linearly related to the environmental indices and genotype DM yields cannot be predicted from estimates of phenotypic means in a single environment. The relatively low degree of linearity confirms indications from other studies that  $G \times E$  interactions are generally not a linear function of the environmental indices when these are defined as the mean of all genotypes tested (Brennan and Byth, 1979). As genotype responses did not depend substantially on environment mean DM yield, environments could not be logically classified or clustered by contrasting levels of mean DM yield (Annicchiarico, 2002).

### Rank correlation coefficients

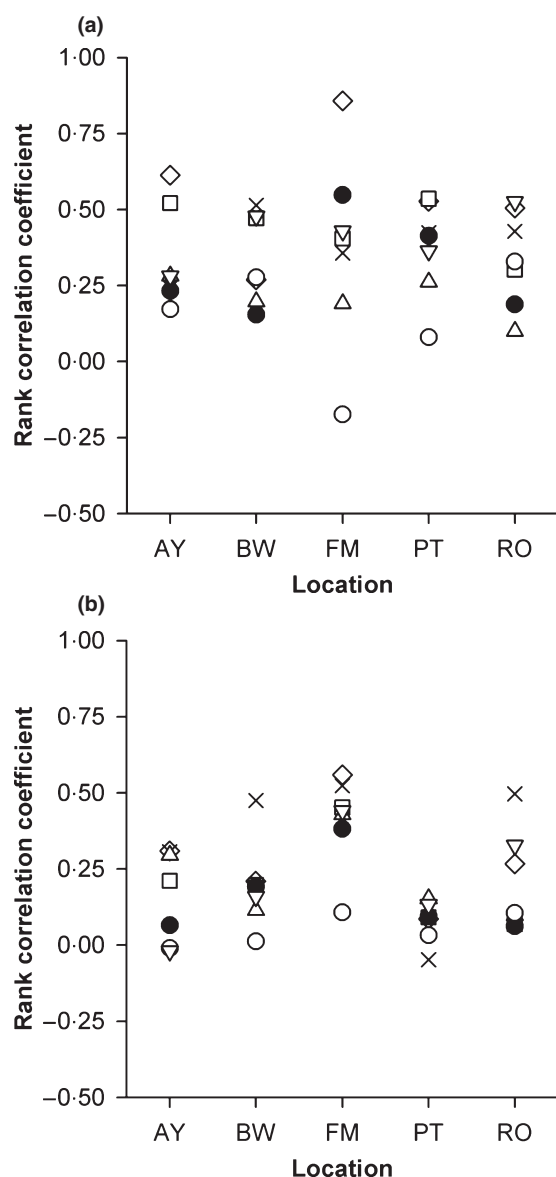
Genotype × age-of-stand and genotype × year-to-year weather interactions for DM yield were due mainly to changes in genotype rankings. Spearman's rank correlations between first- and second-harvest-year data, harvested at the same location in the same calendar year (indicative of age-of-stand effects) (Figure 3) and rank correlations between similar-age swards harvested at the same location but in different calendar years (indicative of annual weather effects) (Figure 4), were low to moderate in magnitude and highly variable, depending on location and harvest period. Taken on the whole, genotype performance in the second harvest year cannot be predicted from first-harvest-year data and genotype performance of similar-age swards cannot be predicted from year to year even at the same location. These results emphasize the need for adequate replication across ages and calendar years in developing



**Figure 3** Mean Spearman's rank correlation coefficients between first- and second-harvest-year genotype yield means (t herbage dry matter ha<sup>-1</sup>) harvested at the same location and in the same calendar year (indicative of age-of-stand effects on genotype rankings independent of year-to-year weather effects). Harvests are represented as follows: ◇, Cut 1; ▽, Cut 2; ×, Cut 3; ○, Cut 4; □, Cut 5; △, Cut 6; ●, annual. See Table 1 for location codes. No valid comparisons for location BC as there was only 1 sowing year.

broad inferences regarding genotype DM yield. The results also call into question the degree to which genotype performance based on the mean of first- and second-harvest-year data correlates with the lifetime (minimum 5 harvest years) genotype performance. Genotypes that have suffered poor persistency in the





**Figure 4** Mean Spearman's rank correlation coefficients between the genotype yield means (t herbage dry matter ha<sup>-1</sup>) of (a) 1-year-old and (b) 2-year-old swards harvested at the same location but in different calendar years (indicative of year-to-year weather variation on genotype rankings independent of age of stand). Harvests are represented as follows:  $\diamond$ , Cut 1;  $\nabla$ , Cut 2;  $\times$ , Cut 3;  $\circ$ , Cut 4;  $\square$ , Cut 5;  $\triangle$ , Cut 6;  $\bullet$ , annual. See Table 1 for location codes. No valid comparisons for location BC as there was only 1 sowing year.

first harvest year are more likely to suffer greater and irreversible plant losses in future years. In the study of Gilliland and Mann (2000), mean rank correlation coefficients between first- and third-year, and between

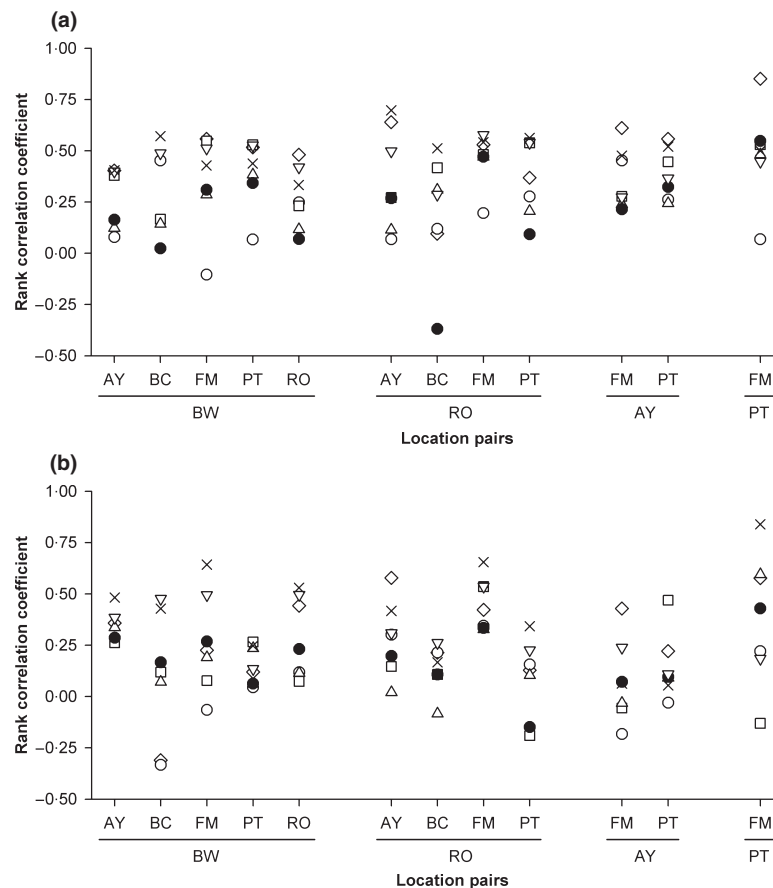
second- and third-year annual DM yields of perennial ryegrass under separate conservation and simulated grazing management trials were low to moderate in magnitude at 0.46 and 0.72 respectively. This suggests that greater weighting should be applied to genotype performance in the second harvest year rather than an equal weighting across both first and second harvest years.

Genotype  $\times$  location interactions for DM yield were also due mainly to changes in genotype rankings. Spearman's rank correlation coefficients between locations, independent of age-of-stand and year-to-year weather effects, were highly variable depending on harvest period but in general low to moderate (Figure 5). For maximum economy of experimentation, it is critical that there is no unnecessary repetition of the locations whose type of environmental response is displayed by other locations. From the rank correlation analysis, it is evident that each location represented a relatively unique environment and all possible combinations of these locations may be considered as potential test environments. The two geographically closest locations, BC and FM, were not compared as they were not sown in the same years.

### Resource allocation

Limited time, land, labour and financial resources mandate that the number of replicates, locations and sowing years must be carefully chosen. An approach to decide on the minimum number or optimum combination of replicates, locations and sowing years is to estimate the genotype LSD under different numbers of these variables. Variables should be allocated in such a way that, for any given cost, the genotype LSD is minimized. A lower genotype LSD maximizes the probability of finding significant differences among genotypes and gives greater confidence that a genotype's DM yield has been correctly characterized.

The relative cost per plot associated with increasing the number of replicates, locations and sowing years varies. Increasing the number of replicates at a given location is usually the cheapest and most feasible option provided that seed is not limiting. It does not take twice as long to cut double the number of replicates. By staggering cutting dates across trials, the current level of equipment and facilities may suffice. Increasing the number of locations is usually the most expensive option as it may require additional equipment and facilities, and significant travelling time. The direct cost of increasing the number of sowing years at a location is, on a cost per plot basis, comparable to increasing the number of replicates. However, the indirect cost is of greater significance. By increasing the number of sowing years, the release of improved cultivars is



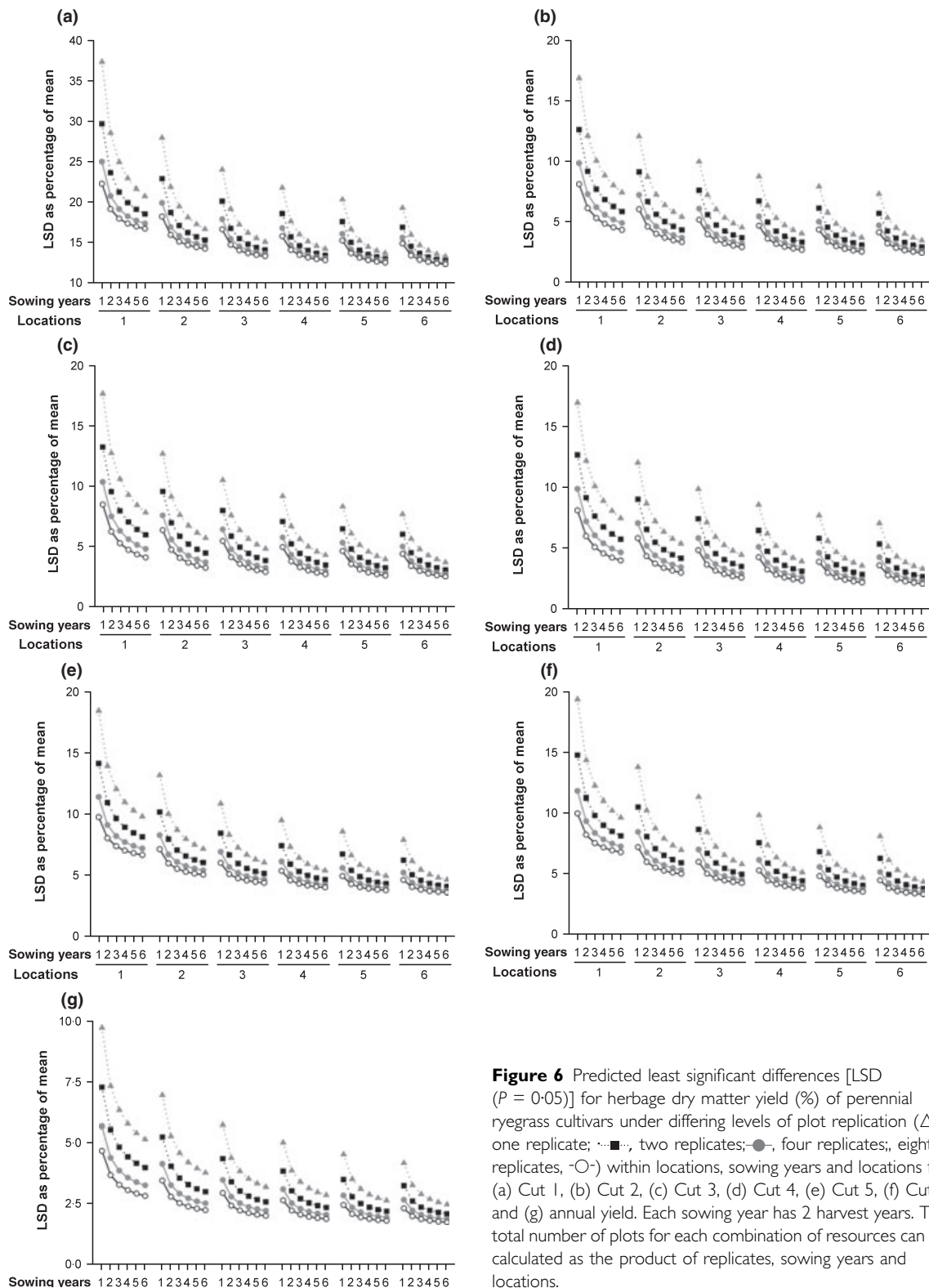
**Figure 5** Mean Spearman's rank correlation coefficients between all possible pairs of locations for the genotype yield means (t forage dry matter ha<sup>-1</sup>) of (a) 1-year-old and (b) 2-year-old swards harvested in the same calendar year (indicative of location effects on genotype rankings independent of age-of-stand and year-to-year weather effects). Harvests are represented as follows: ◇, Cut 1; ▽, Cut 2; ×, Cut 3; ○, Cut 4; □, Cut 5; △, Cut 6; ●, annual. See Table 1 for location codes.

delayed and their commercial life is reduced. For all computations, data were collected from each sowing for 2 harvest years. First, as discussed above, second-harvest-year data may better characterize the lifetime genotype performance than first-harvest-year results. Second, the cost per unit of data in the second harvest year is about half that of the first harvest year (Casler, 1999a). Hence, trials are always conducted for a minimum of 2 harvest years.

The results indicate that, even at its most discriminating with eight replicates, six locations and 6 sowing years, testing could not identify significant differences among genotypes below a difference in DM yield of 12.2, 2.4, 2.5, 2.1, 3.6 and 3.3% for Cuts 1 to 6, respectively, and 1.7% for annual production (Figure 6). The UK national perennial ryegrass testing programme, with eleven locations and two separate managements for conservation and frequent cutting, could not identify significant differences among cul-

tivars below a difference in annual DM yield of 5.9% in the conservation and 3.2% in the frequent cutting managements (Camlin, 1997). The rate of increase in genetic potential for forage DM yield in perennial ryegrass has been estimated as 0.25 to 0.6% per annum (Wilkins and Humphreys, 2003) demonstrating that differences in DM yield for established cultivars compared to candidate cultivars and elite germplasm are likely to be small. Consequently, the risk of misjudgement of the relative merits of genotypes is great and differences among genotypes in DM yield less than the appropriate LSD should be treated with caution.

Increasing the number of replicates, locations and sowing years reduced the LSD among genotype means (Figure 6). However, for all variables the law of diminishing returns sets in and the value of successive additional replicates, locations and sowing years declines progressively. The greater the number of



**Figure 6** Predicted least significant differences [LSD ( $P = 0.05$ )] for herbage dry matter yield (%) of perennial ryegrass cultivars under differing levels of plot replication ( $\Delta$ , one replicate;  $\blacksquare$ , two replicates;  $\bullet$ , four replicates;  $\circ$ , eight replicates;  $\circ$ —, two replicates with error bars) within locations, sowing years and locations for (a) Cut 1, (b) Cut 2, (c) Cut 3, (d) Cut 4, (e) Cut 5, (f) Cut 6 and (g) annual yield. Each sowing year has 2 harvest years. The total number of plots for each combination of resources can be calculated as the product of replicates, sowing years and locations.

replicates per location, the smaller the value of each additional location and sowing year. The greater the number of locations and sowing years, the smaller the value of each additional replicate.

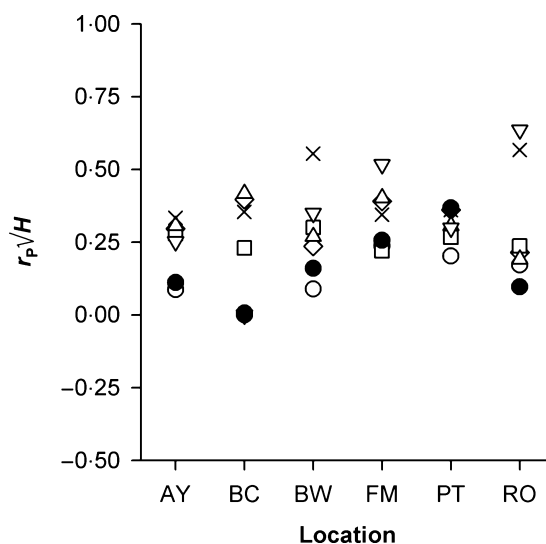
From a practical standpoint, breeders and agronomists usually test using a minimum of two replicates, especially if the number of locations is relatively small ( $\leq 6$ ). If one replicate were to be lost, due to human error, animal effects, weather hazards etc., one replicate would still remain and inferences could still be drawn at that location. Increasing the number of replicates beyond four offered little additional precision. Therefore, the recommended option would be two to four replicates per location. Four replicates and a lesser number of locations and sowing years would offer a comparable efficiency to two replicates but at a reduced cost.

Increasing the number of locations tended to be slightly more efficient than increasing the number of sowing years. However, at two or more locations and sowing years, with a minimum two replicates per location, sowing years could be readily substituted for locations with little effect on efficiency. At four replicates per location, there was little additional precision to be gained from increasing the number of locations and sowing years beyond three each. The optimum allocation of resources would be four replicates per location and either two locations and 3 sowing years or three locations and 2 sowing years. At this level of resources, the mean LSD that may be detected among genotypes is 15.6% for Cut 1, 5.1% for Cuts 2 – 6 and 2.9% for annual production. Both options require twenty-four plots per genotype. The former is cheaper but, provided trials are sown down in consecutive years, requires an extra year. The latter offers marginally better precision, with a relative efficiency of 103% averaged across all harvest periods, but at a greater cost.

The most appropriate option depends on the relative importance of time vs. financial resources. The best allocation of resources will vary from one programme to another. In some programmes, it may well be that resources that could have been allocated to selection for DM yield at an additional location or for an additional sowing year would be better used to select for another trait.

### Selection of locations

Because test locations, particularly those at the national level, are likely to be repeated year after year, a question that naturally follows from the definition of the number of locations to be sampled is: which locations? An accurate selection of test locations is particularly important when a wide adaptation strategy is adopted. Allen *et al.* (1978) proposed that the expression  $r_p\sqrt{H}$  could be used to identify optimum



**Figure 7** Estimates of the optimum selection location for perennial ryegrass trials based on the product of the phenotypic correlation coefficient between the mean dry matter yield of genotypes within a location and the mean dry matter yield of genotypes across all locations, and the square root of broad sense heritability within a location ( $r_p\sqrt{H}$ ), averaged over sowing years. Harvests are represented as follows: ◇, Cut 1; ▽, Cut 2; ×, Cut 3; ○, Cut 4; □, Cut 5; △, Cut 6; ●, annual. See Table 1 for location codes. Results from location BC, based on 1 sowing year, should be treated with caution.

selection locations. In other words, a desirable location should have a high genetic variance, a low environmental variance and be well correlated with the target region (i.e. Ireland). Considering either  $r_p$  or  $\sqrt{H}$  alone may rank locations differently (Barker *et al.*, 1981).

Values of  $r_p\sqrt{H}$  varied widely among harvest periods at the same location and among locations for the same harvest period (Figure 7). Values were generally low to moderate. There was no clear distinction among locations. No location provided a more optimum test location than the other for all harvest periods. Location FM had the highest average  $r_p\sqrt{H}$  across all harvest periods at 0.34 (0.04), providing the most discriminating test location on the average. Average values for locations BW, PT and RO were intermediate at 0.28 (0.06), 0.31 (0.02) and 0.30 (0.08) respectively. The lower standard error for PT, indicating that results were more consistent across harvest periods, favours PT over BW and RO. There was little difference between BW and RO, based on  $r_p\sqrt{H}$ , and choosing between the two locations may be decided on practical considerations. Locations AY and BC had the lowest average  $r_p\sqrt{H}$  at 0.24 (0.04) and 0.20 (0.07) respectively. However, the findings from BC must be treated with caution as data from only 1 sowing year was available. The

estimation of  $r_P\sqrt{H}$  based on a small number of test years may be biased by unusually high or low within-location genotype  $\times$  year interaction or experimental error values (Annicchiarico, 2002).

Theory, however, must be balanced by common sense and practical considerations. Commercial farming has become increasingly associated with the south and east of Ireland as reflected in the economic output of agricultural product per ha (Commings *et al.*, 1999). In parallel, the level of grassland reseeding is greater in the south and east of the country relative to the more extensive north and west. Reseeding is expensive and generally cannot be justified for grassland receiving low or moderate inputs of fertilizer-N (Hopkins *et al.*, 1990). On this basis, locations BW, FM and PT, in the south and east, would be chosen as selection locations over AY and RO, in the north and west, as they are more representative of the major grassland reseeding areas of Ireland.

The order of location desirability, based on a combination of theory and practicality, was FM, PT, BW, RO and AY. If selection were to be practiced at two locations, the optimum combination of locations would be BW and FM. Although  $r_P\sqrt{H}$  suggests PT to be a slightly more efficient location for selection than BW, locations FM and PT are geographically close whereas locations FM and BW are more evenly distributed within the target region representing both the south and east of the country. If selection were to be practiced at three locations, the optimum combination of locations would be BW, FM and PT.

## Conclusions

Significant  $G \times E$  interactions were found for herbage DM yield of perennial ryegrass sward plots in Ireland, necessitating the evaluation of genotypes across multiple locations and years in order to adequately estimate the true genotype merit. Relative differences among genotypes from year to year and location to location were due mainly to changes in genotype rankings. Weather was estimated to have a greater effect than age of stand on annual variation in DM yield. The optimum allocation of resources for a testing programme was estimated at four replicates per location, and either two locations (BW and FM) and 3 sowing years or three locations (BW, FM and PT) and 2 sowing years. An understanding of the nature and causes of  $G \times E$  interactions is necessary to develop a set of recommendations for the experimental design of perennial ryegrass trials.

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